```
FILE 'REGISTRY' ENTERED AT 11:20:28 ON 09 JAN 2003

=> S ENTEROKINASE/CN
L1             1 ENTEROKINASE/CN

=> D

L1   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2003 ACS
RN   9014-74-8  REGISTRY
CN   Peptidase, entero- (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   E.C. 3.4.21.9
CN   E.C. 3.4.4.8
CN     ***Enterokinase***
CN   Enteropeptidase
MF   Unspecified
CI   MAN
LC   STN Files:   AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CABA,
       CAPLUS, CHEMCATS, CHEMLIST, CIN, CSCHEM, EMBASE, IFICDB, IFIPAT, IFIUDB,
       MSDS-OHS, PROMT, TOXCENTER, USPATFULL
     Other Sources:   EINECS**, TSCA**
         (**Enter CHEMLIST File for up-to-date regulatory information)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
             555 REFERENCES IN FILE CA (1962 TO DATE)
               6 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
             557 REFERENCES IN FILE CAPLUS (1962 TO DATE)


FILE 'CAPLUS' ENTERED AT 11:21:01 ON 09 JAN 2003

=> S ENTEROKINASE OR L1
            956 ENTEROKINASE
              6 ENTEROKINASES
            956 ENTEROKINASE
                  (ENTEROKINASE OR ENTEROKINASES)
            557 L1
L2         1049 ENTEROKINASE OR L1

=> S RECOGNITION(W)(SITE OR SEQUENCE);S LIGAND BINDING;S STREPTAVIDIN
          84809 RECOGNITION
            135 RECOGNITIONS
          84870 RECOGNITION
                  (RECOGNITION OR RECOGNITIONS)
         469194 SITE
         438149 SITES
         771607 SITE
                  (SITE OR SITES)
         526606 SEQUENCE
         378095 SEQUENCES
         628759 SEQUENCE
                  (SEQUENCE OR SEQUENCES)
L3        11222 RECOGNITION(W)(SITE OR SEQUENCE)


         232114 LIGAND
         157071 LIGANDS
         317418 LIGAND
                  (LIGAND OR LIGANDS)
         746859 BINDING
           1678 BINDINGS
         747317 BINDING
                  (BINDING OR BINDINGS)
L4        23031 LIGAND BINDING
                  (LIGAND(W)BINDING)


           6229 STREPTAVIDIN
             24 STREPTAVIDINS
L5         6231 STREPTAVIDIN
                  (STREPTAVIDIN OR STREPTAVIDINS)
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=> S L2 AND L3
L6 33 L2 AND L3

=> S L6 AND L4;S L6 AND L5
L7 1 L6 AND L4

L8 2 L6 AND L5

=> S L7,L8
L9 3 (L7 OR L8)

=> S L6 NOT L9
L10 30 L6 NOT L9

=> D L9 1-3 CBIB ABS;D L10 1-30 CBIB ABS

L9 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2003 ACS
2002:965025 ***Enterokinase*** cleavage sequences useful for isolation of fusion proteins. Ley, Arthur Charles; Luneau, Christopher Jon; Ladner, Robert Charles (USA). U.S. Pat. Appl. Publ. US 20020192789 A1 20021219, 67 pp., Cont.-in-part of U. S. Ser. No. 597,321, abandoned. (English). CODEN: USXXCO. APPLICATION: US 2001-884767 20010619. PRIORITY: US 2000-597321 20000619.
AB Novel ***enterokinase*** cleavage sequences are provided. To identify novel ***enterokinase*** cleavage sequences, a substrate phage library, having a diversity of about 2 .times. 108 amino acid sequences, was screened against ***enterokinase*** . The substrate phage library was design to include a peptide-variegated region in the display polypeptide consisting of 13 consecutive amino acids and allowing any amino acid residue except cysteine to occur at each position. The substrate phage library was also characterized by inclusion of an N-terminal tandem arrangement of a linear and a disulfide-constrained ***streptavidin*** ***recognition*** ***sequence*** . The screen was carried through a total of 5 rounds of increasing stringency to obtain phage that could be released by incubation with recombinant light chain ***enterokinase*** after binding to immobilized ***streptavidin*** . Also disclosed are methods for the rapid isolation of a protein of interest present in a fusion protein construct including a novel ***enterokinase*** cleavage sequence of the present invention and a ligand ***recognition*** ***sequence*** for capturing the fusion construct on a solid substrate. Preferred peptides of the present invention (e.g., Asp-Ile-Asn-Asp-Asp-Arg-Xaa) show rates of cleavage (kcat/Km) up to 30-fold that of the known ***enterokinase*** cleavage substrate (Asp)4-Lys-Ile.

L9 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2003 ACS
2001:935666 Document No. 136:66211 Novel ***enterokinase*** cleavage sequences and their use in protein isolation and purification. Ley, Arthur Charles; Luneau, Christopher Jon; Ladner, Robert Charles (Dyax Corp., USA). PCT Int. Appl. WO 2001098366 A2 20011227, 119 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US19539 20010619. PRIORITY: US 2000-597321 20000619.
AB Novel ***enterokinase*** cleavage sequences are provided. Also disclosed are methods for the rapid isolation of a protein of interest present in a fusion protein construct including a novel ***enterokinase*** cleavage sequence of the present invention and a ligand ***recognition*** ***sequence*** for capturing the fusion construct on a solid substrate. Thus, using phage display technol., a no. of novel ***enterokinase*** ***recognition*** ***sequences*** were discovered that provide a highly specific substrate for rapid cleavage by ***enterokinase*** . These show rates of cleavage up to thirty times that of the known ***enterokinase*** cleavage substrate (Asp)4-Lys-Ile.

L9 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2003 ACS

1992:548045 Document No. 117:148045 The cloned platelet thrombin receptor couples to at least two distinct effectors to stimulate phosphoinositide hydrolysis and inhibit adenylyl cyclase. Hung, David T.; Wong, Yung H.; Vu, Thien Khai H.; Coughlin, Shaun R. (Dep. Lab. Med., Univ. California, San Francisco, CA, 94143, USA). Journal of Biological Chemistry, 267(29), 20831-4 (English) 1992. CODEN: JBCHA3. ISSN: 0021-9258.

AB Thrombin both stimulates phosphoinositide hydrolysis and inhibits adenylyl cyclase in a variety of cell types. Whether the cloned human platelet thrombin receptor accounts for both of these signaling events is unknown. The thrombin receptor agonist peptide causes both phosphoinositide hydrolysis and inhibition of adenylyl cyclase in naturally thrombin-responsive CCL-39 cells. To exclude the possibility that the agonist peptide or thrombin itself may activate these pathways via distinct receptors and to circumvent a lack of suitable thrombin receptor-null cells, the authors utilized a designed ***enterokinase*** receptor, a thrombin receptor with its thrombin cleavage ***recognition*** ***sequence*** LDPR replaced by DDDDK, the ***enterokinase*** cleavage ***recognition*** ***sequence*** . Transfection of ***enterokinase*** -unresponsive cells with this construct conferred both ***enterokinase*** -sensitive phosphoinositide hydrolysis and inhibition of adenylyl cyclase. The phosphoinositide hydrolysis response was largely insensitive to pertussis toxin, whereas the adenylyl cyclase response was completely blocked by pertussis toxin. Thus, the cloned thrombin receptor can effect both phosphoinositide hydrolysis and inhibition of adenylyl cyclase via .gtoreq.2 distinct effectors, most likely Gq-like and Gi-like G-proteins.

L10 ANSWER 1 OF 30 CAPLUS COPYRIGHT 2003 ACS

2002:900267 The calcium-binding protein of Entamoeba histolytica as a fusion partner for expression of peptides in Escherichia coli. Reddi, Honey; Bhattacharya, Alok; Kumar, Vijay (Virology Group, International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi, 110 067, India). Biotechnology and Applied Biochemistry, 36(3), 213-218 (English) 2002. CODEN: BABIEC. ISSN: 0885-4513. Publisher: Portland Press Ltd..

AB We describe the construction of an Escherichia coli expression vector, CBP that allows the C-terminal fusion of heterologous proteins to the calcium-binding protein (CaBP) of the parasitic protozoan Entamoeba histolytica. The intrinsic nature of this protein to remain sol. on heat treatment has been exploited in its use as a novel fusion partner. The presence of a histidine tag and an ***enterokinase*** ***recognition*** ***site*** , aid in the affinity purifn. and proteolytic cleavage of the fusion protein. The efficacy of the vector was tested using the preS1 region of the envelope protein of the hepatitis B virus. The CaBP-preS1 fusion protein partitioned in the sol. fraction on heat treatment and this facilitated its rapid purifn.

L10 ANSWER 2 OF 30 CAPLUS COPYRIGHT 2003 ACS

2002:888942 Document No. 138:1068 Method for mass production of peptides via fusion to N-terminal cysteine-containing protein and peptide bond cleavage. Nishimura, Osamu; Suenaga, Masato; Ito, Takashi; Kitada, Chieko (Takeda Chemical Industries, Ltd., Japan). PCT Int. Appl. WO 2002092829 A1 20021121, 88 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (Japanese). CODEN: PIXXD2. APPLICATION: WO 2002-JP4735 20020516. PRIORITY: JP 2001-147341 20010517.

AB A method for producing a peptide on a mass scale by expressing a fusion protein or peptide, wherein a peptide is ligated to the N-end of a N-terminal cysteine-contg. protein or a peptide, and subjecting to the peptide bond cleavage reaction in the amino acid side of the cysteine

residue, is disclosed. A process comprises a combination of the excision of a target peptide with the use of right-handed scissors (S-cyanation reaction) and left-handed scissors (bromocyan treatment, ***enterokinase*** , factor Xa-treatment, etc.) with the tandem repeat method which is useful in synthesizing a peptide (in particular, a low-mol. wt. peptide) in a large amt. with the use of gene recombination techniques. S-cyanation with 2-nitro-5-thiocyanobenzoate, 1-cyano-4-dimethylaminopyridinium, or CN- ion, followed by ammonolysis, or hydrolysis can be used for peptide bond cleavage. Recombinant expression of the human G-protein coupled receptor 8 (GPCR8) ligand peptide and KiSS-1 peptide in E. coli is described.

L10 ANSWER 3 OF 30 CAPLUS COPYRIGHT 2003 ACS
2002:882048 Document No. 137:380935 Expression constructs for the manufacture of peptides as oligomers connected by protease cleavage sites. Dormann, Dirk; Eichner, Wolfram; Sommermeyer, Klaus; Volker, Lang (Fresenius Kabi Deutschland Gmbh, Germany). Ger. Offen. DE 10123348 A1 20021121, 12 pp. (German). CODEN: GWXXBX. APPLICATION: DE 2001-10123348 20010514.

AB Expression constructs for the manuf. of peptides as oligomers connected by peptides that act as proteinase cleavage sites are described. The proteins have the general formula formula A-x1-B(-x2-C1-n)n, where A and B are the same or different oligopeptides with 3 to 100 amino acids; C or C1-n is one or more oligopeptides of discretionary sequence with 3 to 100 amino acids; x1 and x2 is the same or different ***recognition*** ***site*** for peptide cleavage and n is a whole no. larger than 0. A and B may be peptides useful for transport or purifn. of the protein. Furthermore the invention concerns the use of expression constructs for the prodn. of peptides and peptide mixts. as well as the thereby attained peptide mixts. The prepn. of a peptide that could undergo site-specific cleavage by copper at alk. pH is demonstrated.

L10 ANSWER 4 OF 30 CAPLUS COPYRIGHT 2003 ACS
2002:752376 Document No. 137:277775 Protein and cDNA of human cancer cell-specific HLA-F antigen and uses in cancer diagnosis. Egawa, Kohji (Medinet Co., Ltd., Japan; Kimura, Yoshiji). Eur. Pat. Appl. EP 1245675 A1 20021002, 20 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR. (English). CODEN: EPXXDW. APPLICATION: EP 2001-400804 20010328.

AB The invention provides protein and cDNA of human cancer cell-specific HLA-F antigen isolated from U937 cells. This invention provides a method of detecting cancer cells in any organ and irresp. of causes of the tumors. In said method, a new antigenic substance that cancer cells commonly produce in a cancer cell-specific manner is first identified and, then, an antibody produced in response to this antigen is detected in body fluid of cancer patients. Specifically, this is achieved by detecting the anti-HLA-F antibody specific to the cancer cell-specific HLA-F antigen coded by the HLA-F gene.

L10 ANSWER 5 OF 30 CAPLUS COPYRIGHT 2003 ACS
2002:556192 Document No. 137:348212 Expression, purification, and characterization of a biologically active bovine ***enterokinase*** catalytic subunit in Escherichia coli. Yuan, Liu-Di; Hua, Zi-Chun (College of Life Sciences, State Key Laboratory of Pharmaceutical Biotechnology and Institute of Molecular and Cell Biology, Department of Biochemistry, Nanjing University, Nanjing, 210093, Peop. Rep. China). Protein Expression and Purification, 25(2), 300-304 (English) 2002. CODEN: PEXPEJ. ISSN: 1046-5928. Publisher: Elsevier Science.

AB ***Enterokinase*** (EC 3.4.21.9) is a serine proteinase in the duodenum that exhibits specificity for the sequence (Asp)4-Lys. It converts trypsinogen to trypsin. Its high specificity for the ***recognition*** ***site*** makes ***enterokinase*** (EK) a useful tool for in vitro cleavage of fusion proteins. CDNA encoding the catalytic chain of Chinese bovine ***enterokinase*** was cloned and its encoding amino acid sequence is identical to the previously reported sequence although there are two one-base mutations which do not change the encoded amino acid. The EK catalytic subunit cDNA was cloned into plasmid pET32a, and fused downstream to the fusion partner thioredoxin (Trx) and the following DDDDK ***enterokinase*** ***recognition*** ***sequence*** . The recombinant bovine ***enterokinase*** catalytic subunit was expressed in Escherichia coli BL21(DE3), and most products

existed in sol. form. After an in vivo autocatalytic cleavage of the recombinant Trx-EK catalytic domain fusion protein, intact, biol. active EK catalytic subunit was released from the fusion protein. The recombinant intact EK catalytic subunit was purified to homogeneity with a specific activity of 720 AUs/mg protein through ammonium sulfate pptn., DEAE chromatog., and gel filtration. The purified intact EK catalytic subunit has a Km of 0.17 mM, and Kcat is 20.8 s-1. From 100 mL flask culture, 4.3 mg pure active EK catalytic subunits were obtained.

L10 ANSWER 6 OF 30 CAPLUS COPYRIGHT 2003 ACS
2001:284120 Document No. 134:307589 Purification of recombinant proteins fused to multiple epitopes. Brizzard, Bill; Hernan, Ron (Sigma-Aldrich Co., USA). PCT Int. Appl. WO 2001027293 A1 20010419, 51 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US25693 20000920. PRIORITY: US 1999-415000 19991008.

AB The present invention provides novel identification polypeptides contg. multiple copies of an antigenic domain joined in tandem to provide increased sensitivity for the detection and purifn. of target peptides, a cleavable linking sequence and optionally a spacer domain. Further provided are hybrid polypeptide mols. composed of an identification polypeptide and a target peptide which are produced by recombinant DNA technol. and purified using affinity chromatog. using one or more ligands. Accordingly, also provided are DNA expression vectors contg. DNA encoding for identification polypeptides and methods for using such identification polypeptides for the purifn. of target peptides. Thus, a vector was constructed for expression of proteins in mammalian host cells using a modified version of the FLAG expression system, which contains three FLAG sequences in tandem. The first two flag peptides are modified sequences (Asp-Tyr-Lys-Asp-His-Asp) with either a Gly or Ile spacer domain between the two sequences. Proteolytic ***recognition*** ***sites*** are also included for cleavage by ***enterokinase*** , thrombin, or factor Xa. The p3XFLAG-CMV-7 expression vector contains the human cytomegalovirus promoter region necessary for constitutive expression of cloned genes in many mammalian cell lines, as well as the Kozak consensus sequence, a multiple cloning site, and the SV40 origin of replication. A 10-fold increase in detection limit of the triple FLAG-bacterial alk. phosphatase was obsd. compared to the single FLAG-BAP fusion protein, and 500 pg of purified 2XFlag bacterial alk. phosphatase could be detected with exposures as short as 1 min. A 10-fold increase detection was also demonstrated in both dot blot and ELISA assay.

L10 ANSWER 7 OF 30 CAPLUS COPYRIGHT 2003 ACS
2000:615648 Document No. 134:13775 Expression of a Kallikrein-like Protease from the Snake Venom: Engineering of Autocatalytic Site in the Fusion Protein to Facilitate Protein Refolding. Hung, Chin-Chun; Chiou, Shyh-Horng (Institute of Biochemical Sciences, Coll. Sci., Natl. Taiwan Univ., Taiwan). Biochemical and Biophysical Research Communications, 275(3), 924-930 (English) 2000. CODEN: BBRCA9. ISSN: 0006-291X. Publisher: Academic Press.

AB In order to circumvent the difficulty encountered in the expression and purifn. of the recombinant products in E. coli system, we have developed a novel and facile method of removing the polyhistidine tag from target proteins after heterologous gene expression. The expression of a serine protease (Tm-5) from Taiwan habu (Trimeresurus mucrosquamatus) is taken as an exemplar to illustrate the basic rationales and protocols involved. In place of an ***enterokinase*** ***recognition*** ***site*** , a polyhistidine tag linked to an autocatalyzed site based on cleavage specificity of the serine protease flanking on the 5'-end of Tm-5 clone sequence was engineered before protein expression in E. coli system. Renaturation of the fusion protein after expression revealed that the recombinant protease had refolded successfully from the inclusion bodies. Upon autocleavage of the expressed protease, the polyhistidine tag with addnl. amino acid residues appended to the N-terminus of the coding sequence is found to be removed accordingly. The protein expressed and

purified by this new strategy possesses a mol. wt. of approx. 28,000 in accord with the expected value for this venom protease. Further characterization of the recombinant protein employing a variety of techniques which include immunoblot anal., RP-HPLC, ESI-MS, and N-terminal amino acid sequencing all shows indistinguishable properties to those of the isolated native protease. Most noteworthy is that the recombinant Tm-5 protease also exhibits amidase activity against N-benzoyl-Pro-Phe-Arg-p-nitroanilide, a unique and strict substrate for native Tm proteases reported previously. (c) 2000 Academic Press.

L10 ANSWER 8 OF 30 CAPLUS COPYRIGHT 2003 ACS
2000:273190 Document No. 133:295074 Construction and characterization of a novel recombinant single-chain variable fragment antibody against Western equine encephalitis virus. Long, Melissa C.; Jager, Scott; Mah, Dave C. W.; Jebailey, Lellean; Mah, Maria A.; Masri, Saad A.; Nagata, Les P. (Medical Countermeasures Section, Defence Research Establishment Suffield, Medicine Hat, AB, T1A 8K6, Can.). Hybridoma, 19(1), 1-13 (English) 2000. CODEN: HYBRDY. ISSN: 0272-457X. Publisher: Mary Ann Liebert, Inc..
AB A novel recombinant single-chain fragment variable (scFv) antibody against Western equine encephalitis virus (WEE) was constructed and characterized. Using antibody phage display technol., a scFv was generated from the WEE specific hybridoma, 10B5 E7E2. The scFv was fused to a human heavy chain IgG1 const. region (CH1-CH3) and contained an intact 6 His tag and ***enterokinase*** ***recognition*** ***site*** (RS10B5huFc). The RS10B5huFc antibody was expressed in E. coli and purified by affinity chromatog. as a 70-kDa protein. The RS10B5huFc antibody was functional in binding to WEE antigen in indirect enzyme-linked immunosorbent assays (ELISAs). Furthermore, the RS10B5huFc antibody was purified in proper conformation and formed multimers. The addn. of the human heavy chain to the scFv replaced effector functions of the mouse antibody. The Fc domain was capable of binding to protein G and human complement. The above properties of the RS10B5huFc antibody make it an excellent candidate for immunodetection and immunotherapy studies.

L10 ANSWER 9 OF 30 CAPLUS COPYRIGHT 2003 ACS
1999:718875 Document No. 131:348774 Tandem fluorescent protein constructs and their preparation for enzyme assays. Tsien, Roger Y.; Heim, Roger; Cubitt, Andrew (The Regents of the University of California, USA; Aurora Biosciences Corporation). U.S. US 5981200 A 19991109, 33 pp., Cont.-in-part of U.S. Ser. No. 594,575. (English). CODEN: USXXAM. APPLICATION: US 1997-792553 19970131. PRIORITY: US 1996-594575 19960131.
AB This invention provides tandem fluorescent protein construct including a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties. The donor and acceptor moieties exhibit fluorescence resonance energy transfer which is eliminated upon cleavage. The constructs are useful in enzymic assays. Mutant green fluorescent proteins (GFPs) were created by mutagenesis of the Aequorea victoria GFP. Polyhistidine tagged tandem green and blue fluorescent proteins were recombinantly constructed having an inserted peptide sequence including cleavage ***recognition*** ***sites*** for many proteases. Cleavage expts. were done with trypsin, ***enterokinase*** and calpain.

L10 ANSWER 10 OF 30 CAPLUS COPYRIGHT 2003 ACS
1999:461847 Document No. 131:224110 S. pombe expression vector with 6.times.(His) tag for protein purification and potential for ligation-independent cloning. Hosfield, Tanya; Lu, Quinn (Stratagene Cloning Systems, La Jolla, CA, USA). BioTechniques, 27(1), 58-60 (English) 1999. CODEN: BTNQDO. ISSN: 0736-6205. Publisher: Eaton Publishing Co..
AB A new plasmid vector, pESP-2, was constructed and tested for gene expression and protein prodn. in Schizosaccharomyces pombe. This vector contains the S. pombe inducible nmt1 promoter for high-level gene expression and the 6.times.(His) affinity tag for protein purifn. The vector contains a multiple cloning site with unique restriction enzyme sites and ligation-independent cloning (LIC) sites. The LIC sites enable direction cloning of a PCR product immediately downstream of an ***enterokinase*** ***recognition*** ***site*** . Thus, polypeptides without extraneously added amino acids can be obtained by removing the purifn. tag with ***enterokinase*** after purifn. of the recombinant 6.times.(His) fusion protein.

L10 ANSWER 11 OF 30 CAPLUS COPYRIGHT 2003 ACS
1999:226539 Document No. 131:68807 A new series of pET-derived vectors for high efficiency expression of Pseudomonas exotoxin-based fusion proteins. Matthey, Barbel; Engert, Andreas; Klimka, Alexander; Diehl, Volker; Barth, Stefan (Laboratory of Immunotherapy, Dep. I of Internal Medicine, University Hospital of Cologne, Cologne, 50931, Germany). Gene, 229(1-2), 145-153 (English) 1999. CODEN: GENED6. ISSN: 0378-1119. Publisher: Elsevier Science B.V..

AB Recombinant immunotoxins (rITs) are highly specific anti-tumor agents composed of monoclonal antibody fragments or other specific carriers coupled to plant or bacterial toxins. A major problem in the purifn. of rITs is the low periplasmic yield in currently available expression systems. Thus, the aim of this study was the development of a new bacterial expression system for high-level prodn. of rITs. We constructed a series of pET-based vectors for pelB-directed periplasmic secretion or cytoplasmic prodn. under the control of the T7lac promoter. Expression in Escherichia coli BL21 (DE3)pLysS allowed a tightly regulated iso-Pr .beta.-d-thiogalactopyranoside (IPTG) induction of protein synthesis. An ***enterokinase*** -cleavable poly-histidine cluster was introduced into this setup for purifn. by affinity chromatog. A major modification resulted from the insertion of a specifically designed multiple cloning site. It contains only rare restriction enzyme ***recognition*** ***sites*** used for cloning of Ig variable region genes, as well as unique SfiI and NotI restriction sites for directed insertion of single-chain variable fragments (scFv) available from established bacteriophage systems. For this purpose, we deleted two naturally occurring internal SfiI consensus sites in a deletion mutant of Pseudomonas aeruginosa exotoxin A (ETA'). Each single structural element of the new vector (promoter, leader sequence, purifn. tag, scFv sequence, selectable marker, and toxin gene) was flanked by unique restriction sites allowing simple directional substitution. The fidelity of IPTG induction and high-level expression were demonstrated using an anti-CD30 scFv (Ki-4) fused to ETA'. These data confirm a bacterial vector system esp. designed for efficient periplasmic expression of ETA'-based fusion toxins.

L10 ANSWER 12 OF 30 CAPLUS COPYRIGHT 2003 ACS
1999:201355 Document No. 131:70195 Influence of the Amino Acid Residue Downstream of (Asp)4Lys on ***Enterokinase*** Cleavage of a Fusion Protein. Hosfield, Tanya; Lu, Quinn (Stratagene Cloning Systems, Inc., La Jolla, CA, 92037, USA). Analytical Biochemistry, 269(1), 10-16 (English) 1999. CODEN: ANBCA2. ISSN: 0003-2697. Publisher: Academic Press.

AB We have studied the cleavage efficiency of the protease ***enterokinase*** (EK) using the novel vector pESP4. Plasmid pESP4 is a yeast expression vector equipped with ligation-independent cloning sites, a GST purifn. tag, and a FLAG epitope tag. EK is used to cleave the FLAG and GST tags leaving the protein of interest without any extraneously added amino acids. We have found that EK is relatively permissive of the amino acid residue downstream of the ***recognition*** ***sequence*** (the P'1 position). This makes EK an ideal choice to use as a protease to cleave any protein of interest cloned within the pESP4 yeast expression vector. (c) 1999 Academic Press.

L10 ANSWER 13 OF 30 CAPLUS COPYRIGHT 2003 ACS
1998:296766 Document No. 129:64697 A thioredoxin fusion protein of VanH, a D-lactate dehydrogenase from Enterococcus faecium: cloning, expression, purification, kinetic analysis, and crystallization. Stoll, Vincent S.; Manohar, A. Vaito; Gillon, Wanda; Macfarlane, Emma L. A.; Hynes, Rosemary C.; Pai, Emil F. (Department of Biochemistry, University of Toronto, Toronto, ON, M5S 1A8, Can.). Protein Science, 7(5), 1147-1155 (English) 1998. CODEN: PRCIEI. ISSN: 0961-8368. Publisher: Cambridge University Press.

AB The gene encoding the vancomycin resistance protein VanH from Enterococcus faecium, a D-lactate dehydrogenase, has been cloned into a thioredoxin expression system (pTRxFus) and expressed as a fusion protein. The use of several other expression systems yielded only inclusion bodies from which no functional protein could be recovered. Expts. to remove the thioredoxin moiety by ***enterokinase*** cleavage at the engineered ***recognition*** ***site*** under a variety of conditions resulted in nonspecific proteolysis and inactivation of the protein. The intact fusion protein was, therefore, used for kinetic studies and crystn.

trials. It has been purified to greater than 90% homogeneity by ammonium sulfate pptn. followed by Ph Sepharose chromatog. Based on kcat/KM for pyruvate, it is 20% as active as native VanH. Michaelis consts. for NADPH, NADH, and pyruvate, of .apprx.3.5 .mu.M, 19.0 .mu.M, and 1.5 mM, resp., were comparable to those reported for the native VanH (Bugg TDH et al., 1991, Biochem. 30:10408-10415). Like native VanH, max. activity of the fusion protein requires the presence of an anion (phosphate or acetate); however, in addn., a strongly reducing environment is needed for optimal efficacy. Competitive inhibition consts. for ADP-ribose, NAD+, and oxamate have also been detd. Crystn. by hanging drop vapor diffusion produced two different crystal forms, one hexagonal and the other tetragonal. Flash-frozen crystals of the tetragonal form diffracted to 3.0 .ANG. resoln. at a synchrotron radiation source.

L10 ANSWER 14 OF 30 CAPLUS COPYRIGHT 2003 ACS
1997:707262 Document No. 128:10990 Influence of a NH2-terminal extension on the activity of KTX2, a K+ channel blocker purified from Androctonus australis scorpion venom. Legros, Christian; Feyfant, Eric; Sampieri, Francois; Rochat, Herve; Bougis, Pierre E.; Martin-Eauclaire, Marie-France (Laboratoire de Biochimie, Ingenierie des Proteines, UMR 6560 du Centre National de la Recherche Scientifique, Institut Federatif Jean Roche, Faculte de Medecine Nord, Boulevard Pierre Dramard, Marseille, 13916/20, Fr.). FEBS Letters, 417(1), 123-129 (English) 1997. CODEN: FEBLAL. ISSN: 0014-5793. Publisher: Elsevier.
AB A cDNA encoding a short polypeptide blocker of K+ channels, kaliotoxin 2 (KTX2), from the venom of the North African scorpion Androctonus australis was expressed in the periplasmic space of Escherichia coli. KTX2 was produced as a fusion protein with the maltose binding protein followed by the ***recognition*** ***site*** for factor Xa or ***enterokinase*** preceding the first amino acid residue of the toxin. The fully refolded recombinant KTX2 (rKTX2) was obtained (0.15-0.30 mg/L of culture) and was indistinguishable from the native toxin according to chem. and biol. criteria. An N-extended analog of KTX2 exhibiting three addnl. residues was also expressed. This analog had 1000-fold less affinity for the 125I-kaliotoxin binding site on rat brain synaptosomes than KTX2. Conformational models of KTX2 and its mutant were designed by amino acid replacement using the structure of agitoxin 2 from Leiurus quinquestriatus as template, to try to understand the decrease in affinity for the receptor.

L10 ANSWER 15 OF 30 CAPLUS COPYRIGHT 2003 ACS
1996:696040 Document No. 126:30362 Production of recombinant growth hormone releasing factor via post-translational C-terminal .alpha.-amidation. Henriksen, D. B.; Stout, J. S.; Partridge, B. E.; Holmquist, B.; Wagner, F. W. (BioNebraska, Inc., Lincoln, NE, 68524, USA). Peptides: Chemistry, Structure and Biology, Proceedings of the American Peptide Symposium, 14th, Columbus, Ohio, June 18-23, 1995, Meeting Date 1995, 651-652. Editor(s): Kaumaya, Pravin T. P.; Hodges, Robert S. Mayflower Scientific: Kingswinford, UK. (English) 1996. CODEN: 63NTAF.
AB A process involving 3 enzymic steps and a 2-stage HPLC purifn. has been used to prep. multi-gram amts. of injectable grade GRF(1-44)NH2. GRF(1-44) contg. a C-terminal Ala extension has been expressed in Escherichia coli as a fusion protein linked to human carbonic anhydrase (HCA) through an interlinking peptide contg. ***recognition*** ***sites*** for thrombin and ***enterokinase*** . The Ala extension provides an enzymic ***recognition*** ***site*** for post-translational .alpha.-amidation. Incorporation of the peptide onto HCA affords protection against proteolytic degrdn.

L10 ANSWER 16 OF 30 CAPLUS COPYRIGHT 2003 ACS
1996:27944 Document No. 124:84989 Production of a recombinant bovine ***enterokinase*** catalytic subunit in the methylotrophic yeast Pichia pastoris. Vozza, Laura A.; Wittwer, Leonard; Higgins, David R.; Purcell, Thomas J.; Bergseid, Mark; Collins-Racie, Lisa A.; LaVallie, Edward R.; Hoeffler, James P. (Invitrogen Corp., San Diego, CA, 92121, USA). Bio/Technology, 14(1), 77-81 (English) 1996. CODEN: BTCHDA. ISSN: 0733-222X. Publisher: Nature Publishing Co..
AB The authors describe the heterologous expression of a 26.3 kDa protein contg. the catalytic domain of bovine ***enterokinase*** (EKL) in the methylotrophic yeast Pichia pastoris. A highly active protein is secreted and glycosylated, and it has the native amino-terminus of EKL. The cDNA

encoding EKL was cloned with the KEX2 protease cleavage site following the .alpha. mating factor prepro secretion signal from Saccharomyces cerevisiae. The secreted EKL was easily purified from the few native proteins found in the P. pastoris fermn. supernatant, using ion exchange and affinity chromatog. The yield of the purified EKL was 6.3 mg per L of fermn. culture. This is significantly higher than previous reports of expressions in E. coli and COS cells. The ability of this highly specific protease to cleave immediately after the carboxyl-terminal residue of the (Asp)4-Lys ***recognition*** ***sequence*** allows regeneration of native amino-terminal residues of recombinant proteins. Its application is demonstrated by the removal of thioredoxin (TrxA), and polyhistidine fusion partners from proteins of interest.

L10 ANSWER 17 OF 30 CAPLUS COPYRIGHT 2003 ACS

1995:934612 Document No. 124:46816 Versatile, multi-featured plasmids for high-level expression of heterologous genes in Escherichia coli: overproduction of human and murine cytokines. Mertens, Nico; Remaut, Erik; Fiers, Walter (Laboratory of Molecular Biology, University of Gent, B-9000, Ghent, Belg.). Gene, 164(1), 9-15 (English) 1995. CODEN: GENED6. ISSN: 0378-1119. Publisher: Elsevier.

AB We describe the construction, expression characteristics and some applications of a versatile dual-promoter expression plasmid for heterologous gene expression in Escherichia coli which contains both .lambda. pL and PT7 promoters. Furthermore, the plasmid is optimized to allow the expression of mature coding sequences without compromising the strength of the highly efficient PT7 or of the T7g10 ribosome-binding site. The effect of the naturally occurring RNA loops at both the 5' and 3' ends of the T7g10 mRNA on expression was also examd. A double T7 RNA polymerase transcription terminator was inserted to ensure more reliable transcription termination and a higher expression level of the preceding gene. Further improvements involve a clockwise orientation of the promoters to minimize read-through transcription from plasmid promoters, a largely extended multiple cloning site, an antisense phage T3 promoter and a phage f1-derived, single-stranded replication origin. Variants of this plasmid allow for the prodn. of fusion proteins with part of T7g10, a hexahistidine peptide and an ***enterokinase*** ***recognition*** ***site*** . The potential of these expression vectors is demonstrated by comparing the expression levels of a no. of mammalian cytokines (human tumor necrosis factor, human immune interferon, human and murine interleukins 2, murine interleukin 4 and murine fibroblast interferon), using these expression plasmids.

L10 ANSWER 18 OF 30 CAPLUS COPYRIGHT 2003 ACS

1995:774399 Document No. 123:167663 Production of recombinant bovine ***enterokinase*** catalytic subunit in Escherichia coli using the novel secretory fusion partner DsbA. Collins-Rcie, Lisa A.; McColgan, James M.; Grant, Kathleen L.; DiBlasio-Smith, A.; McCoy, John M.; LaVallie, Edward R. (Genetics Institute, Cambridge, MA, 02140, USA). Bio/Technology, 13(9), 982-7 (English) 1995. CODEN: BTCHDA. ISSN: 0733-222X. Publisher: Nature Publishing Co..

AB ***Enterokinase*** (EK) is a heterodimeric serine protease which plays a key role in initiating the proteolytic digestion cascade in the mammalian duodenum. The enzyme acts by converting trypsinogen to trypsin via a highly specific cleavage following the pentapeptide ***recognition*** ***sequence*** (Asp)4-Lys. This stringent site specificity gives EK great potential as a fusion protein cleavage reagent. Recently, a cDNA encoding the catalytic (light) chain of bovine ***enterokinase*** (EKL), was identified, characterized, and transiently expressed in mammalian COS cells. The authors report here the prodn. of EKL in Escherichia coli by a novel secretory expression system that utilizes E. coli DsbA protein as an N-terminal fusion partner. The EKL cDNA was fused in-frame to the 3'-end of the coding sequence for DsbA, with the two domains of the fusion protein sepd. by a linker sequence encoding an ***enterokinase*** ***recognition*** ***site*** . Active, processed recombinant EKL (rEKL) was generated from this fusion protein via an autocatalytic cleavage reaction. The enzymic properties of the bacterially produced rEKL were indistinguishable from the previously described COS-derived enzyme. Both forms of rEKL were capable of cleaving peptides, polypeptides and trypsinogen with the same specificity exhibited by the native heterodimeric enzyme purified from bovine duodena. Interestingly, rEKL activated trypsinogen poorly relative to the native

heterodimeric enzyme, but was superior in its ability to cleave artificial fusion proteins contg. the (Asp)4-Lys ***recognition*** ***sequence*** .

L10 ANSWER 19 OF 30 CAPLUS COPYRIGHT 2003 ACS
1995:621604 Document No. 123:28218 Enzymatic method for modification of recombinant polypeptides. Wagner, Fred W.; Stout, Jay; Henriksen, Dennis; Partridge, Bruce; Manning, Shane (Bionebraska, Inc., USA). PCT Int. Appl. WO 9503405 A2 19950202, 70 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1994-US8125 19940719. PRIORITY: US 1993-95162 19930720.
AB An enzymic method is provided for the formation of a recombinant polypeptide which has been modified at the C-terminal end through the use of a transpeptidation process. The method is suitable for modifying recombinant polypeptides of any source including those which may be com. available, those derived from recombinant single copy or multi-copy polypeptide constructs, or those derived from single or multi-copy recombinant fusion proteins constructs. The transpeptidation reaction involves contacting an endopeptidase enzyme with a recombinant polypeptide to substitute and addn. unit, of one or more acids, for leaving unit, linked to a core polypeptide through a cleavage site recognized by the endopeptidase enzyme. Recombinant polypeptides derived from multi-copy polypeptide constructs may be cleaved from the multi-copy polypeptide at the N-terminal and C-terminal ends and simultaneously undergo substitution of the leaving unit by the desired addn. unit. The invention utilizes known and newly discovered cleavage ***recognition*** ***sites*** of effectuate the desired modification products. Prepn. of C-terminally amidated glucagon like peptide 1 and growth hormone releasing factor using trypsin and thrombin, resp., as an endopeptidase was demonstrated.

L10 ANSWER 20 OF 30 CAPLUS COPYRIGHT 2003 ACS
1994:427987 Document No. 121:27987 Introduction of arbitrary sequences into genes by use of class IIs restriction enzymes. Beck, Reinhard; Burtscher, Helmut (Boehringer Mannheim Res. Cent., Penzberg, D-82372, Germany). Nucleic Acids Research, 22(5), 886-7 (English) 1994. CODEN: NARHAD. ISSN: 0305-1048.
AB The authors wanted to insert a DNA sequence coding for four histidine residues and an ***enterokinase*** cleavage site between the signal sequence and the mature sequence of human placental alk. phosphatase for purifn. The authors added the ***enterokinase*** ***recognition*** ***site*** in order to allow regeneration of the authentic N-terminus of the enzyme. Here the authors describe a convenient way to achieve this, using class IIs restriction endonucleases. Class IIs restriction endonucleases cut DNA several nucleotides away from their ***recognition*** ***site*** irresp. of the intervening sequence. This can be exploited to generate arbitrary sticky ends for in-frame fusion of DNA sequences, combining two PCR reactions with a simple cloning step. The authors designed two oligonucleotide primers (ON2, ON3) contg. the desired sequences, a SapI restriction site and some addnl. nucleotides (including extra restriction endonuclease cleavage sites for other purposes). The whole procedure takes about 2 to 3 days from PCR to transformation of cells. This method works with fragments of a wide range of sizes, fragments can be inserted, deleted or fused regardless of their individual size differences. The protocol is very simple, using std. PCR conditions for introduction of a class IIs restriction enzyme ***recognition*** ***site*** and easy ligation of the sticky ends created by cleavage at the "transient" site.

L10 ANSWER 21 OF 30 CAPLUS COPYRIGHT 2003 ACS
1994:268195 Document No. 120:268195 Combinatorial polypeptide antigens. Crea, Roberto (Creagen, Inc., USA). PCT Int. Appl. WO 9400151 A1 19940106, 49 pp. DESIGNATED STATES: W: AU, CA, JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1993-US5899 19930618. PRIORITY: US 1992-900123 19920618.
AB A set of polypeptide antigens having amino acid sequences derived from amino acid sequences of a population of variants of a protein, or a

portion thereof, is produced by (1) selecting a protein, or a portion thereof, which exhibits a population of N variants, represented by the formula A1A1A3 ... An-2An-1An, where An is an amino acid occurring at amino acid position n of the protein, or portion thereof; (2) detg. the no. of times Onaa each type of amino acid occurs at each amino acid position n in the N variants; (3) calcg. the frequency of occurrence (Onaa/N)n of each type of amino acid at each amino acid position n in the N variants; and (4) generating a set of polypeptide antigens having amino acid sequences represented substantially by the formula A'1A'2A'3 ... A'n-2A'n-1A'n, where A'n is defined as an amino acid type which occurs at greater than a selected frequency at the corresponding amino acid position in the N variants. Thus, a vaccine directed to envelope glycoprotein gp120 of HIV-1, which shows marked sequence diversity between isolates, was produced by tabulating the frequency of occurrence of amino acids at each of the positions in the V3 loop (residues 303-338), deriving a degenerate oligonucleotide sequence encoding .gtoreq.90% of the variants, synthesizing this oligonucleotide by ligating 6 shorter oligonucleotides, amplifying by PCR to a V3 loop gene library, fusing in-frame with an ***enterokinase*** cleavage ***recognition*** ***sequence*** , cloning and expression in Escherichia coli, and removal of protein A sequences from the fusion protein with ***enterokinase*** .

L10 ANSWER 22 OF 30 CAPLUS COPYRIGHT 2003 ACS

1993:487512 Document No. 119:87512 Interaction cloning: identification of a helix-loop-helix zipper protein that interacts with c-Fos. Blanar, Michael A.; Rutter, William J. (Hormone Res. Inst., Univ. California, San Francisco, CA, 94143, USA). Science (Washington, DC, United States), 256(5059), 1014-18 (English) 1992. CODEN: SCIEAS. ISSN: 0036-8075.

AB A facile method for isolating genes that encode interacting proteins has been developed with a polypeptide probe that contains an amino-terminal extension with ***recognition*** ***sites*** for a monoclonal antibody, a specific endopeptidase, and a site-specific protein kinase. This probe, contg. the basic region-leucine zipper dimerization motif of c-Fos, was used to screen a cDNA library. A cDNA that encoded a member of the basic-helix-loop-helix-zipper (bHLH-Zip) family of proteins was isolated. The cDNA-encoded polypeptide FIP (Fos interacting protein) bound to oligonucleotide probes that contained DNA binding motifs for other HLH proteins. When cotransfected with c-Fos, FIP stimulated transcription of an AP-1-responsive promoter.

L10 ANSWER 23 OF 30 CAPLUS COPYRIGHT 2003 ACS

1993:162448 Document No. 118:162448 Expression vectors encoding an affinity peptide for rapid purification of fusion proteins by affinity chromatography. Blanar, Michael A.; Kaelin, William G. (Dana Farber Cancer Institute, USA). PCT Int. Appl. WO 9303157 A1 19930218, 89 pp. DESIGNATED STATES: W: CA, JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1992-US6187 19920724. PRIORITY: US 1991-736847 19910729.

AB Expression vectors that allow synthesis of a protein with an affinity peptide for rapid purifn. of the protein are described. The construct is under control of a strongly inducible promoter and includes a convenient multicloning site and encodes a cleavage site for a specific proteinase (e.g., for thrombin or factor Xa) to allow removal of the affinity label. A sequence for a site that can be conveniently labeled may also be included. A specific vector including coding sequences for glutathione-S-transferase, a thrombin cleavage site, and a phosphorylation ***recognition*** ***site*** for a cAMP-dependent protein kinase is described. The plasmid was constructed by std. methods and used to manuf. domains of the retinoblastoma susceptibility gene product that bind viral antigens. The proteins were manufd. in Escherichia coli and purified by affinity chromatog. on glutathione-Sepharose. After binding, the protein was enzymically phosphorylated and eluted with reduced glutathione. The phosphorylated protein retained the expected binding properties towards large T antigen and the E1A gene product. The use of the labeled protein to screen expression libraries for genes for proteins with which it interacts was demonstrated.

L10 ANSWER 24 OF 30 CAPLUS COPYRIGHT 2003 ACS

1993:123060 Document No. 118:123060 Production of authentic human proapolipoprotein A-I in Escherichia coli: strategies for the removal of the amino-terminal methionine. Moguilevsky, Nicole; Varsalona, Francesca;

Guillaume, Jean Paul; Gilles, Pascal; Bollen, Alex; Roobol, Kees (Univ. Brussels, Nivelles, B-1400, Belg.). Journal of Biotechnology, 27(2), 159-72 (English) 1993. CODEN: JBITD4. ISSN: 0168-1656.

AB Several methods were compared with respect to the prodn. of authentic, N-terminal methionine-free proapolipoprotein A-I in engineered E. coli bacteria. A 1st approach consisted of treating the purified methionylated recombinant protein with an aminopeptidase purified from Aeromonas proteolytica. A 2nd series of strategies was based on the construction of proapo A-I-encoding cassettes carrying built-in ***recognition*** ***sites*** suitable for specific in vitro cleavage of the products with kallikrein or ***enterokinase*** . Along the same line, a fusion between ubiquitin and proapo A-I was produced in E. coli with the prospect of achieving post-purifn. cleavage with yeast ubiquitin hydrolase. Finally, proapo A-I was fused to the signal peptide of the bacterial outer membrane protein OmpA, aiming at an in situ conversion to authentic proapo A-I during secretion to the bacterial periplasm. These 5 systems, the OmpA signal peptide system and, to a lesser extent, the one involving the fusion to ubiquitin were the most efficient in yielding authentic proapo A-I from engineered E. coli.

L10 ANSWER 25 OF 30 CAPLUS COPYRIGHT 2003 ACS

1988:623979 Document No. 109:223979 Cloning and expression of cDNA for human apolipoprotein or variant in Escherichia coli. Lorenzetti, Rolando; Monaco, Lucia; Soria, Marco; Palomba, Raffaele; Isacchi, Antonella; Sarmientos, Paolo (Farmitalia Carlo Erba S.p.A., Italy). Eur. Pat. Appl. EP 267703 A1 19880518, 30 pp. DESIGNATED STATES: R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1987-309318 19871021. PRIORITY: GB 1986-25435 19861023.

AB The cDNA for human apolipoprotein A1 (apoA1) and its variants are cloned and expressed in Escherichia coli. Plasmid pLM8 was constructed contg. the protein A gene fused to the mature human apoA1 cDNA. The binding affinity of the recombinant protein to J774 or Fao cell surface receptors was 3.5-4.9 .times. 10-8 M, compared with 2.8-3.0 .times. 10-8 M for high-d. lipoprotein purified from human plasma.

L10 ANSWER 26 OF 30 CAPLUS COPYRIGHT 2003 ACS

1988:217269 Document No. 108:217269 High-yield expression of modified human granulocyte colony-stimulating factor gene in yeast and Escherichia coli. Cerretti, Douglas Pat; Cosman, David John; Gillis, Stephen; Mochizuki, Diane Yukiko; March, Carl Jack; Price, Virginia Lee; Tushinski, Robert J.; Urdal, David Lloyd (Immunex Corp., USA). Eur. Pat. Appl. EP 243153 A2 19871028, 38 pp. DESIGNATED STATES: R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1987-303509 19870422. PRIORITY: US 1986-856643 19860422; US 1986-931458 19861114.

AB Human granulocyte colony-stimulating factor (hG-CSF) derivs. are recombinantly produced in high yields in yeast and Escherichia coli hosts. Plasmid pBC102.K22 was constructed contg. a site-specifically mutagenized hG-CSF gene (having the codon for arginine at position 22 replaced with that for lysine such that a KEX2 protease-sensitive site is eliminated) linked at the 5'-end via a KEX2 ***recognition*** ***site*** to an .alpha.-factor leader sequence and a sequence encoding an antigenic peptide capable of cleavage by bovine ***enterokinase*** . Yeast transformed with pBC102.K22 showed 5-fold higher expression than yeast transformed with vector contg. native hG-CSF protein gene.

L10 ANSWER 27 OF 30 CAPLUS COPYRIGHT 2003 ACS

1987:552858 Document No. 107:152858 Human atrial natriuretic factor and its manufacture. Hobden, Adrian; Dykes, Colin (Glaxo Group Ltd., UK). Brit. UK Pat. Appl. GB 2180539 A1 19870401, 24 pp. (English). CODEN: BAXXDU. APPLICATION: GB 1986-18123 19860724. PRIORITY: GB 1985-18753 19850724.

AB A fused DNA encoding a hybrid protein comprising human atrial natriuretic factor (ANF) polypeptide, a linker protein contg. a proteolytic enzyme ***recognition*** ***site*** , and a carrier polypeptide is constructed. The hybrid protein avoids the degrdn. of the short human ANF polypeptide by the proteases of the transformed host cells, e.g. Escherichia coli. Recombinant plasmid PTCX2 contg. a Tac promoter, a transcription terminator, the chloramphenicol acetyltransferase (CAT) structural gene, and XbaI and XhoI restriction sites was ligated with an ANF-Xba oligomer which contained an ANF-coding sequence and an XbaI ***recognition*** ***site*** to obtain expression vector pTCAX21.

The fusion protein was purified from a lysate of cells transformed with pTCAX21, cleaved with Staphylococcus aureus V8 protease, and chromatographed to yield complete ANF.

L10 ANSWER 28 OF 30 CAPLUS COPYRIGHT 2003 ACS
1987:208794 Document No. 106:208794 Plasmid vector containing a signal for specific cleavage of chimeric proteins. Preparation of [Leu5] enkephalin with the aid of enteropeptidase. Dobrynin, V. N.; Boldyreva, E. F.; Filippov, S. A.; Chuvpilo, S. A.; Korobko, V. G.; Vorotyntseva, T. I.; Bessmertnaya, L. Ya.; Mikhailova, A. G.; Amerik, A. Yu.; Antonov, V. K. (M. M. Shemyakin Inst. Bioorg. Chem., Moscow, USSR). Bioorganicheskaya Khimiya, 13(1), 119-21 (Russian) 1987. CODEN: BIKHD7. ISSN: 0132-3423.
AB Plasmid vector pEK1 was constructed such that it contains a enteropeptidase (EC 3.4.21.9) [***9014-74-8***] ***recognition*** ***site*** -encoding region within a .beta.-galactosidase [9031-11-2] gene. Synthetic DNA encoding [Leu5]enkephalin [58822-25-6] was fused to the .beta.-galactosidase gene at this site after restriction by KpnI, and the recombinant plasmid pEK-ENK was used to transform Escherichia coli. Transformant clones formed the fused protein at 30% of total protein yield when induced with isopropylthio-.beta.-D-galactopyranoside. After ion-exchange fractionation the chimeric protein was cleaved with enteropeptidase to yield [Leu5]enkephalin.

L10 ANSWER 29 OF 30 CAPLUS COPYRIGHT 2003 ACS
1987:28521 Document No. 106:28521 Improved expression using fused genes providing for protein product. Cousens, Lawrence S.; Tekamp-Olson, Patricia A.; Shuster, Jeffrey R.; Merryweather, James P. (Chiron Corp., USA). Eur. Pat. Appl. EP 196056 A2 19861001, 36 pp. DESIGNATED STATES: R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1986-104066 19860325. PRIORITY: US 1985-717209 19850328.
AB A method for enhancing the prodn. of heterologous proteins in fungi by recombinant DNA techniques involves fusion of a gene encoding a heterologous protein produced in large amt. and in stable form in the host to a sequence encoding a desired heterologous protein, where the hybrid proteins produced are joined by a selectively cleavable linkage. Plasmid pYASI1 was constructed which contains the human superoxide dismutase gene fused to the amino terminus of the human proinsulin gene, with a methionine codon at the junction, under the control of the hybrid inducible ADH2-GAP promoter and the GAP terminator. The fusion protein produced by yeast transformants accounts for .gtoreq.10% of the total cell protein. After cleavage of the hybrid protein at the methionine junction using CNBr and formic acid in water, the proinsulin was converted to its S-sulfonate form in the presence of urea, Na sulfite, and Na tetrathionate, and was purified on an ion-exchange column. Proinsulin-S-sulfonate obtained was 90% pure, and the yield was 150 mg protein/124 g yeast.

L10 ANSWER 30 OF 30 CAPLUS COPYRIGHT 2003 ACS
1978:402253 Document No. 89:2253 Structural basis for the specific activation of human cationic trypsinogen by human enteropeptidase. Brodrick, James W.; Largman, Corey; Hsiang, Myrtle W.; Johnson, Janice H.; Geokas, Michael C. (Enzymol. Res. Lab., Martinez Veterans Hosp., Martinez, CA, USA). Journal of Biological Chemistry, 253(8), 2737-42 (English) 1978. CODEN: JBCHA3. ISSN: 0021-9258.
AB Human pancreatic cationic trypsinogen was activated by human enteropeptidase much more readily than bovine trypsinogen, the ratios kcat/Km being 330 and 11 mM-1 s-1, resp. Conversely, porcine enteropeptidase activated bovine trypsinogen much more rapidly (kcat/Km = 630 mM-1 s-1) than human cationic trypsinogen (kcat/Km = 2.4 mM-1 s-1). The primary structure of the activation region of human cationic trypsinogen was investigated in an attempt to elucidate the basis for these findings. The sequence of the 1st 12 residues at the N-terminus of human cationic trypsinogen was Asp-Lys-Ile-Val-Gly-Gly-Tyr-Asn-Cys-Glu-Glu-Asn. Furthermore, the activation peptide derived from human cationic trypsinogen was isolated and shown to be the dipeptide, Asp-Lys. This result is in contrast to the Val-(Asp)4-Lys activation peptide from bovine trypsinogen and demonstrates that human cationic trypsinogen does not contain the (Asp)4 sequence present in many other mammalian trypsinogens. It is proposed that the high degree of specificity for activation of human cationic trypsinogen by human enteropeptidase is due to the preferential

recognition of the novel activation peptide sequence in the human zymogen. Thus, these 2 functionally related proteins, cationic trypsinogen and enteropeptidase, may have evolved in a parallel manner in the human lineage.

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=> E LEY A/AU
=> S E3,E4,E13-E16
             6 "LEY A"/AU
             5 "LEY A C"/AU
             5 "LEY ARTHUR"/AU
            22 "LEY ARTHUR C"/AU
             9 "LEY ARTHUR CHARLES"/AU
             1 "LEY ARTHUR CHARLES JR"/AU
L11         48 ("LEY A"/AU OR "LEY A C"/AU OR "LEY ARTHUR"/AU OR "LEY ARTHUR
               C"/AU OR "LEY ARTHUR CHARLES"/AU OR "LEY ARTHUR CHARLES JR"/AU)

=> E LUNEAU C/AU
=> S E4-E6
             1 "LUNEAU CHRISTOPHER"/AU
             7 "LUNEAU CHRISTOPHER J"/AU
             2 "LUNEAU CHRISTOPHER JON"/AU
L12         10 ("LUNEAU CHRISTOPHER"/AU OR "LUNEAU CHRISTOPHER J"/AU OR "LUNEAU
                CHRISTOPHER JON"/AU)

=> E LADNER R/AU
=> S E3,E4,E6-E8
             2 "LADNER R"/AU
             9 "LADNER R C"/AU
             2 "LADNER ROBERT"/AU
            20 "LADNER ROBERT C"/AU
            32 "LADNER ROBERT CHARLES"/AU
L13         65 ("LADNER R"/AU OR "LADNER R C"/AU OR "LADNER ROBERT"/AU OR "LADN
               ER ROBERT C"/AU OR "LADNER ROBERT CHARLES"/AU)

=> S L11,L12,L13
L14        104 (L11 OR L12 OR L13)

=> S L14 AND L2
L15          2 L14 AND L2

=> S L15 NOT L6
L16          0 L15 NOT L6
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	L #	Hits	Search Text	DBs
1	L1	2607	ENTEROKINASE	USPAT ; US-PG PUB
2	L2	12933	RECOGNITION ADJ (SITE OR SEQUENCE)	USPAT ; US-PG PUB
3	L3	1158	L1 AND L2	USPAT ; US-PG PUB
4	L4	658	L1 SAME L2	USPAT ; US-PG PUB
5	L5	572	L1 WITH L2	USPAT ; US-PG PUB
6	L6	46	L1 ADJ L2	USPAT ; US-PG PUB